

# The Effects of $\gamma$ -tocopherol Administration on Pretibial Edema in Young Women with Premenstrual Syndrome

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Gamma-tocopherol is largely metabolized to 2,7,8-trimethyl-2-(2'-carboxyethyl)-6-hydroxychroman ( $\gamma$ -CEHC), which has natriuretic activity that is mediated via inhibition of 70 pS ATP-sensitive K<sup>+</sup> channels in the thick ascending limb of the loop of Henle. However, the effects of  $\gamma$ -tocopherol administration on edema are unclear. To determine the effects of  $\gamma$ -tocopherol administration on pretibial edema, we measured urinary  $\gamma$ -CEHC concentration by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) after administration of  $\gamma$ -tocopherol. Twenty young women who had a history of pretibial edema due to premenstrual syndrome were randomly divided into two groups. The  $\gamma$ -tocopherol group received 4 " $\gamma$ -tocopherol capsules" (each containing 100mg of  $\gamma$ -tocopherol), and the control group received 4 "placebo capsules" (each containing 250mg of soybean oil) per day for 7 days. Urinary sodium and potassium secretion and urine volume did not increase after cessation of  $\gamma$ -tocopherol administration, yet the degree of pretibial edema improved in all participants in the  $\gamma$ -tocopherol group. The serum  $\gamma$ -tocopherol concentration significantly increased in the  $\gamma$ -tocopherol group. Urinary excretion of  $\gamma$ -CEHC significantly increased after  $\gamma$ -tocopherol administration. Our results suggest that orally administered  $\gamma$ -tocopherol on renal sodium handling is not apparent, but  $\gamma$ -tocopherol is a precursor of prolonged natriuresis of  $\gamma$ -CEHC and may be effective for edema.

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## Introduction

The vitamin E family is a group of tocopherols and tocotrienols that has four homologs ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) differing in the methyl substitutions on the chromanoxyl ring, and the saturation of the phytyl tail. These vitamin E homologs, especially  $\alpha$ - and  $\gamma$ -tocopherol, are equally well absorbed from the intestine, transported by chylomicrons in lymph, and incorporated into hepatic cells. Alpha-tocopherol transfer protein ( $\alpha$ -TTP) specifically selects  $\alpha$ -tocopherol in the liver from among all the incoming tocopherols for incorporation into very low density lipoprotein (VLDL) and transfer to the blood. Therefore,  $\alpha$ -tocopherol is the main tocopherol circulating in the blood and detectable in tissues, and is thought to have the highest biological activity of the tocopherols, despite higher dietary intakes of  $\gamma$ -tocopherol than  $\alpha$ -tocopherol.<sup>1-5</sup> However,  $\gamma$ -tocopherol has been recently discussed as having a role beyond that of an antioxi-

dant. Gamma-tocopherol is largely metabolized to 2,7,8-trimethyl-2-(2'-carboxyethyl)-6-hydroxychroman ( $\gamma$ -CEHC) as a result of truncation of the phytyl tail, mediated initially by cytochrome P450-dependent  $\omega$ -oxidation, followed by  $\beta$ -oxidation.<sup>6</sup> Catabolism of  $\gamma$ -tocopherol to  $\gamma$ -CEHC, followed by glucuronide conjugation and urinary excretion, is a major pathway for elimination of  $\gamma$ -tocopherol in humans.<sup>7,8</sup> Gamma-CEHC has a natriuretic activity that is mediated via inhibition of 70 pS ATP-sensitive K<sup>+</sup> channels in the thick ascending limb of the loop of Henle<sup>9,10</sup> as well as anti-inflammatory activity.<sup>11</sup> It would be interesting to determine the relationship between  $\gamma$ -CEHC and volume-expanded status such as pregnancy, congestive heart failure, liver cirrhosis, and chronic renal failure because  $\gamma$ -CEHC might play an important role in substances exhibiting a prolonged natriuresis.<sup>10,12</sup> However, the bioavailability of  $\gamma$ -tocopherol and the metabolism of vitamin E after chronic administration of  $\gamma$ -tocopherol to humans with edema are not well

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understood.<sup>13</sup>

Premenstrual syndrome (PMS) is a collection of heterogeneous symptoms that are attributed to hormonal fluctuations and that vary among individuals, but the etiology remains unclear. These symptoms arise or are exacerbated during the luteal phase of the menstrual cycle and ameliorate after the onset of menses.<sup>14</sup> The most prevalent severe symptoms are emotional and behavioral (irritability, mood lability, depressed moods, anxiety, impulsivity, social friction, and feelings of "loss of control"), cognitive (decreased concentration), and physical (bloating, breast swelling and tenderness, general aches, and fatigue).<sup>15</sup> Bloating or peripheral edema is one of these symptoms, because female hormones such as progesterone and estrogens modulate the renal handling of sodium.<sup>16</sup> Fluid retention during the luteal phase may be due to a relative deficiency of atrial natriuretic factor and a lower threshold for arginine vasopressin.<sup>17</sup> Spironolactone, a steroid receptor antagonist and a diuretic to remove the excess fluid, is reported to improve this symptom as well as irritability, depression, breast tenderness, and food craving.<sup>18</sup>

To determine the effects of  $\gamma$ -tocopherol administration on pretibial edema and urinary excretion of  $\gamma$ -CEHC in young women with PMS, we measured  $\gamma$ -CEHC in human urine by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) after administration of  $\gamma$ -tocopherol.

## Materials and Methods

### Study protocol

A total of 20 young women who had a history of pretibial edema due to PMS (ranging in age from 20 to 25 years, mean  $20.9 \pm 1.6$  years) were enrolled in the present study. The definition of PMS in the present study was as follows: (1) The symptoms include emotional and behavioral (irritability, mood lability, depressed moods, anxiety, impulsivity, social friction, and feelings of "loss of control"), cognitive (decreased concentration), and physical (bloating, breast swelling and tenderness, general aches, and fatigue). (2) These symptoms arise or are exacerbated approximately 2 to 10 days before next menses and ameliorate after the onset of menses. Other major symptoms included irritability in 8 women, depressed moods in 4 women, sleepiness in 2 women, breast swelling in 2 women, diarrhea in 3 women, constipation in 3 women, and abdominal or lumbar pain in 16 women. None of them were receiving medication or taking regular vitamin supplements. We randomly divided (by the numbered container method) the 20 women into two groups: one group that was given  $\gamma$ -tocopherol capsules ( $n=10$ ; the  $\gamma$ -tocopherol group) and a control group ( $n=10$ ). The  $\gamma$ -tocopherol group received 4 " $\gamma$ -tocopherol capsules" (each containing 100mg of  $\gamma$ -tocopherol and 150mg of soybean oil) per day for 7 days, and the control group received 4 "placebo capsules" (each containing 250mg of soybean oil) per day for 7 days. Two capsules were given within 30 min after breakfast and the other two within 30 min after supper. These capsules were obtained from

Eisai Food & Chemical Co. (Tokyo, Japan). Before (day 0, 14 days before the expected next onset of menstruation) and after cessation of administration (day 7), body weight and percentage body fat measurements were performed using a bipedal bioimpedance instrument (Karada Scan HBF-352, OMRON Co., Kyoto, Japan), and the degree of pretibial edema was judged by a physician (M.M.) who was blinded to the treatment protocol as follows: (-), absent; ( $\pm$ ), slightly present; (+), apparently present. After an overnight fast, blood was obtained from each patient before capsule administration (day 0) and after cessation of capsule administration (day 7). A 24-hour urine sample was also obtained from each participant on the days of blood testing. Furthermore, a dietary study on all participants was performed before capsule administration (day 0) and after cessation of capsule administration (day 7), and dietary intake levels of calories, protein,  $\gamma$ -tocopherol, sodium, and potassium were calculated using calculation software (Excel Eiyokun, Kenpakusha, Tokyo, Japan). This study was performed according to the principles of the Declaration of Helsinki; the study protocol was approved by the Ethical Committee of Siebold University of Nagasaki, and informed consent was obtained from all participants.

### Determination of urine volume, urinary and serum creatinine, sodium, and potassium concentrations, and creatinine clearance

Urine volume was measured after urine collection. Urinary and serum creatinine levels were measured using a commercial kit (Creatinine-Test Wako; Wako Pure Chemical Industries, Osaka, Japan) based on the Jaffe reaction. Urinary and serum sodium and potassium levels were measured by an automatic analyzer (SRL, Inc, Tokyo, Japan). Creatinine clearance (Ccr) was calculated using urinary creatinine concentration (U, mg/dL), urine volume (V, mL/day), serum creatinine concentration (S, mg/dL), body surface area (A, m<sup>2</sup>), and the standard body surface area of a Japanese adult (1.73, m<sup>2</sup>) based on the following formula:  $Ccr \text{ (mL/min)} = (U \times V / S \times 1,440) \times 1.73/A$ .

### Determination of serum $\alpha$ - and $\gamma$ -tocopherol concentrations by HPLC

The serum concentrations of  $\alpha$ - and  $\gamma$ -tocopherol were measured by high-performance liquid chromatography (HPLC). A volume of 50 $\mu$ L of serum was diluted in 800 $\mu$ L of methanol. After addition of 100 $\mu$ L of 1 mg/L  $\delta$ -tocopherol as an internal standard, the sample was vortex-mixed and centrifuged at 6,000 r.p.m. for 1 min. Then, 50 $\mu$ L of the supernatant was injected into the HPLC system.<sup>19</sup> The HPLC system consisted of a PU-1580 pump (JASCO Corporation, Tokyo, Japan), AS-1555-10 autosampler (JASCO), FP-1520 fluorescence detector ( $\lambda_{exc} = 295\text{nm}$ ,  $\lambda_{em} = 330\text{nm}$ , JASCO), and a Kantomightsyl RP-18GP column (4.6mm x 150mm, 5 $\mu$ m, Kanto Chemical Co., Tokyo, Japan). The mobile phase was pure methanol, and the flow rate was set at 0.1 mL/min.

### Determination of urinary $\gamma$ -CEHC concentration by HPLC

The urinary concentration of  $\gamma$ -CEHC was measured by HPLC with electrochemical detection (HPLC-ECD). One hundred  $\mu$ L of  $\beta$ -glucuronidase (type VII-A, from *E. coli*, 25,000 units, Sigma, St. Louis, MO, USA) solution (1,000 U/mL in 0.1 mol/L acetate buffer), 100 $\mu$ L of ascorbate solution (10mg/mL), and 20 $\mu$ L of butylated hydroxytoluene (BHT) solution (12.5  $\mu$ g/mg in ethanol) were added in 1 mL of urine sample, and incubated for 2 hours at 37 °C to hydrolyze the conjugate. After cooling on ice, 50 $\mu$ L of 6 mol/L HCl and 2 mL of diethylether were added, and the mixture was centrifugated at 3,500 r.p.m. for 10 min. Thereafter, 1 mL of the diethylether layer was collected and evaporated to dryness using nitrogen gas. The residue was dissolved in 200 $\mu$ L of BHT solution, and filtered using a 0.02 $\mu$ m-4mm Millipore filtration system (Billerica, MA, USA). A 10- $\mu$ L aliquot was injected into the HPLC-ECD system. A known amount of  $\gamma$ -CEHC solution donated from Eisai Co. (Tokyo, Japan) was used as a control. The HPLC system consisted of a PU-1580 pump (JASCO), PL-300 damper (Eicom Co., Kyoto, Japan), AS-1555-10 autosampler (JASCO), CO-1560 column thermostat (JASCO), ECD-300 electrochemical detector (Eicom), HTEC-500 system (Eicom) with an applied potential of 550mV, and a Mightysil RP-18 GP column (2.0mm x 250mm, 5 $\mu$ m, Kanto Chemical Co.). The column temperature was set at 37 °C, and the mobile phase was 32.5% (v/v) acetonitrile containing 50 mmol/L NaClO<sub>4</sub> and 0.5 mmol/L EDTA-2Na. The flow rate was set at 0.2 mL/min.

### Statistical analysis

All values were expressed as mean  $\pm$  standard error (SE).

Differences between groups were tested for statistical significance using a Student's t-test for paired and unpaired data.  $P < 0.05$  was considered significant.

## Results

### Clinical features, laboratory data, and daily intake of calories, protein, sodium, and potassium before capsule administration and after cessation of capsule administration

Of the 10  $\gamma$ -tocopherol group participants, one woman was excluded from the study population because she hurt her leg during the study period. Participant's age (mean, 20.7 vs 21.1 years old), body weight (mean, 53.3 vs 52.6 kg), percentage body fat (mean, 25.0 vs 25.9%), 24-hour urine volume (mean, 776 vs 900 mL), creatinine clearance (mean, 103.1 vs 74.0 mL/min), urinary sodium (mean, 2.8 vs 2.6 g/day) and potassium levels (mean, 1.3 vs 1.3 g/day), serum sodium (mean, 143 vs 142 mEq/L) and potassium levels (mean, 4.1 vs 4.0 mEq/L), and dietary intake levels of calories (mean 1,663 vs 1,663 kcal), protein (mean, 62.3 vs 57.2 g),  $\gamma$ -tocopherol (mean, 11.4 vs 7.8 mg), sodium (mean, 3,438 vs 3,052 mg), and potassium (mean, 1,729 vs 2,238 mg) were not significantly different between the control group (n=10) and the  $\gamma$ -tocopherol group (n=9), and also between before capsule administration (day 0) and after cessation of capsule administration (day 7) ( $p > 0.05$ ) (Table 1).

### Degree of pretibial edema

In the control group, the degree of pretibial edema was improved from (+) to ( $\pm$ ) in 2 participants, from (+) to (-) in 1 partici-

**Table 1.** Clinical features, laboratory data, and daily intake of calories, protein, sodium, and potassium in participants in the control and  $\gamma$ -tocopherol groups

Feature	Control group (n=10)		$\gamma$ -tocopherol group (n=9)	
	Day 0	Day 7	Day 0	Day7
Age (year)	20.7 $\pm$ 1.3		21.1 $\pm$ 1.8	
Body weight (kg)	53.3 $\pm$ 1.9	52.9 $\pm$ 1.9	52.6 $\pm$ 1.6	52.7 $\pm$ 1.8
Percentage body fat (%)	25.0 $\pm$ 0.8	25.3 $\pm$ 0.9	25.9 $\pm$ 0.8	26.5 $\pm$ 0.8
24-hour urine volume (mL)	776 $\pm$ 100	774 $\pm$ 117	900 $\pm$ 154	886 $\pm$ 124
Creatinine clearance (mL/min)	103.1 $\pm$ 31.2	97.1 $\pm$ 20.1	74.0 $\pm$ 8.4	84.0 $\pm$ 23.0
Urinary sodium (g/day)	2.8 $\pm$ 0.4	2.6 $\pm$ 0.3	2.6 $\pm$ 0.2	2.4 $\pm$ 0.3
Urinary potassium (g/day)	1.3 $\pm$ 0.1	1.5 $\pm$ 0.2	1.3 $\pm$ 0.2	1.5 $\pm$ 0.2
Serum sodium (mEq/L)	143 $\pm$ 0	142 $\pm$ 0	142 $\pm$ 0.4	142 $\pm$ 0.6
Serum potassium (mEq/L)	4.1 $\pm$ 0.1	3.9 $\pm$ 0.1	4.0 $\pm$ 0.1	4.0 $\pm$ 0.1
Daily energy intake (kcal)	1,663 $\pm$ 140	1,529 $\pm$ 173	1,663 $\pm$ 115	1,622 $\pm$ 176
Daily protein intake (g)	62.3 $\pm$ 5.5	56.8 $\pm$ 7.3	57.2 $\pm$ 6.1	56.3 $\pm$ 7.1
Daily $\gamma$ -tocopherol intake (mg)	11.4 $\pm$ 4.9	11.7 $\pm$ 2.0	7.8 $\pm$ 1.8	6.6 $\pm$ 1.1
Daily sodium intake (mg)	3,438 $\pm$ 356	3,347 $\pm$ 252	3,052 $\pm$ 390	3,584 $\pm$ 284
Daily potassium intake (mg)	1,729 $\pm$ 205	1,748 $\pm$ 163	2,238 $\pm$ 290	1,903 $\pm$ 179

Data are expressed as mean  $\pm$  SE.

**Table 2.** The degree of pretibial edema in young women with premenstrual syndrome before (day 0) and after administration of "placebo" or " $\gamma$ -tocopherol capsules" (day 7)

Degree of pretibial edema	Day 0	Day 7	Number of participants
Control group (n=10)			
	(+)	(+)	1
	(+)	( $\pm$ )	2
	(+)	(-)	1
	( $\pm$ )	( $\pm$ )	2
	( $\pm$ )	(-)	4
$\gamma$ -tocopherol group (n=9)			
	(+)	( $\pm$ )	2
	(+)	(-)	1
	( $\pm$ )	(-)	6

The degree of pretibial edema was judged as follows: (-), absent; ( $\pm$ ), slightly present; (+), apparently present.

pant, and from ( $\pm$ ) to (-) in 4 participants, whereas pretibial edema was not improved in 3 participants. In the  $\gamma$ -tocopherol group, the degree of pretibial edema was improved in all 9 participants: from (+) to ( $\pm$ ) in 2 participants, from (+) to (-) in 1 participant, and from ( $\pm$ ) to (-) in 6 participants (Table 2).

#### Serum $\alpha$ - and $\gamma$ -tocopherol concentration.

The serum  $\alpha$ -tocopherol concentration in the  $\gamma$ -tocopherol group ( $0.6 \pm 0.1$  mg/dL) was significantly lower than that in the control group ( $1.1 \pm 0.1$  mg/dL) after cessation of  $\gamma$ -tocopherol administration (day 7) ( $p < 0.01$ ), while there was no significant difference in serum  $\alpha$ -tocopherol concentration before  $\gamma$ -tocopherol administration (day 0) between the control group ( $1.0 \pm 0.05$  mg/dL) and the  $\gamma$ -tocopherol group ( $0.9 \pm 0.04$  mg/dL) (Figure 1). The serum  $\gamma$ -tocopherol concentration in the  $\gamma$ -tocopherol group ( $588.1 \pm 69.2$   $\mu$ g/dL) was significantly higher than that in the control group ( $244.5 \pm 123.0$   $\mu$ g/dL) on day 7 ( $p < 0.01$ ), while there was no significant difference in serum  $\gamma$ -tocopherol concentration on day 0 between the control group ( $98.1 \pm 14.5$   $\mu$ g/dL) and the  $\gamma$ -tocopherol group ( $95.4 \pm 11.6$   $\mu$ g/dL) (Figure 2).

#### Urinary $\gamma$ -CEHC concentration

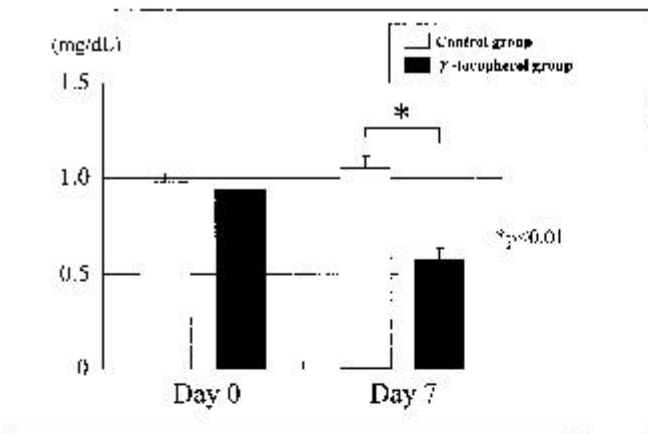
The peak of  $\gamma$ -CEHC was clearly detected on a HPLC chromatogram of urine samples in our assay conditions (Figure 3). The urinary excretion of  $\gamma$ -CEHC in the  $\gamma$ -tocopherol group ( $551.5 \pm 62.4$   $\mu$ g/mL) was significantly higher than that in the control group ( $38.7 \pm 9.1$   $\mu$ g/mL) on day 7 ( $p < 0.01$ ), while there was no significant difference in urinary  $\gamma$ -CEHC concentration on day 0 between the control group ( $54.0 \pm 9.4$   $\mu$ g/mL) and the  $\gamma$ -tocopherol group ( $56.2 \pm 11.7$   $\mu$ g/mL) (Figure 4).

## Discussion

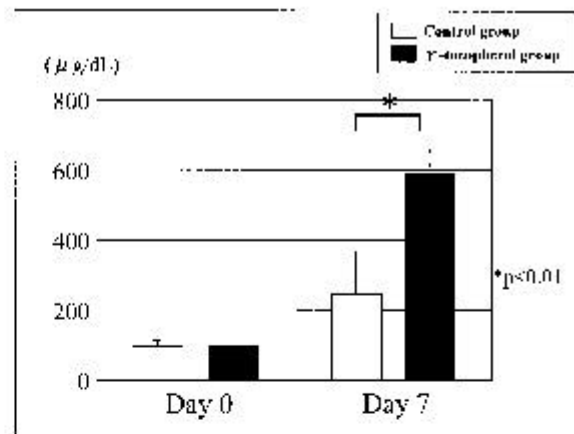
Alpha- and/or  $\gamma$ -CEHC concentrations have been measured in human urine, human plasma, rat bile, and tissue samples by HPLC or liquid chromatography-mass spectrometry (LC-MS), but this area is still fairly new and evolving.<sup>20-23</sup> In the present study, we successfully measured the urinary concentration of  $\gamma$ -CEHC in humans by HPLC-ECD using a small sample volume (1mL) and rapid turnaround time, and confirmed that urinary excretion of  $\gamma$ -CEHC significantly increased after  $\gamma$ -tocopherol administration.

In the present study, urinary sodium and potassium secretion and urine volume were not increased on day 7 in the  $\gamma$ -tocopherol group, yet the degree of pretibial edema was improved in all 9 participants in the  $\gamma$ -tocopherol group. Uto et al.<sup>12</sup> reported that  $\gamma$ -tocopherol increased the urine volume and accelerated sodium excretion into rat urine with a high sodium intake, but sodium excretion did not change with a normal sodium intake. They further reported that this effect was observed in a dose-dependent manner.<sup>24</sup> On the other hand, Yoshikawa et al.<sup>13</sup> reported that urinary sodium excretion was significantly increased but the urine volume did not change one week after the cessation of  $\gamma$ -tocopherol administration in healthy adult males with a normal sodium intake. The reason for the discrepancy between Yoshikawa's results and ours is unclear. In the present study, urinary sodium and potassium secretion and urine volume were measured only just after the cessation of  $\gamma$ -tocopherol administration. These parameters should have been monitored during and a couple of weeks after the administration periods to determine the natriuretic effect of  $\gamma$ -CEHC.

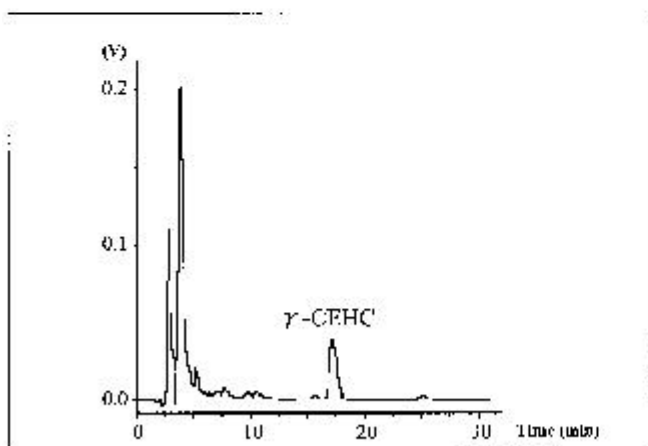
It would be interesting to determine the relationship between urinary  $\gamma$ -CEHC concentration and the effect on pretibial edema. Unfortunately, such investigation could not be conducted in the present study, because the degree of pretibial edema was improved not only in the  $\gamma$ -tocopherol group (9/9) but also in the control group (7/10). This may be partially due to the fact that daily  $\gamma$ -



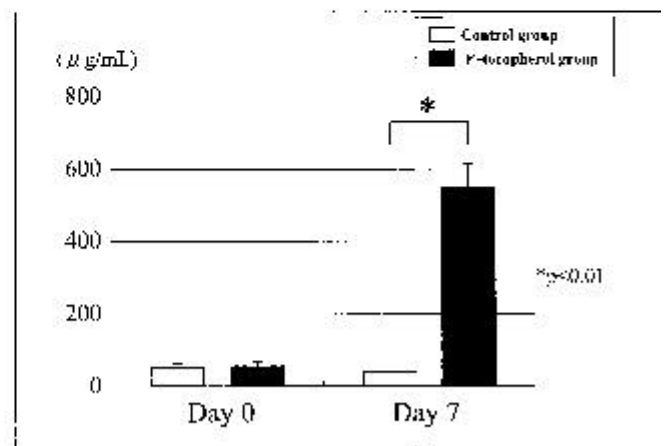
**Figure 1.** The serum concentration of  $\alpha$ -tocopherol in young women with premenstrual syndrome before (day 0) and after administration of "placebo" or " $\gamma$ -tocopherol capsules" (day 7).



**Figure 2.** The serum concentration of  $\gamma$ -tocopherol in young women with premenstrual syndrome before (day 0) and after administration of "placebo" or " $\gamma$ -tocopherol capsules" (day 7).



**Figure 3.** Representative HPLC-ECD chromatogram of urinary  $\gamma$ -CEHC, as confirmed by authentic standard of  $\gamma$ -CEHC solution.



**Figure 4.** The urinary concentration of  $\gamma$ -CEHC in young women with premenstrual syndrome before (day 0) and after administration of "placebo" or " $\gamma$ -tocopherol capsules" (day 7).

tocopherol intake tended to be high in the control group (day 0;  $11.4 \pm 4.9$  mg/day, day 7;  $11.7 \pm 2.0$  mg/day) and low in the  $\gamma$ -tocopherol group (day 0;  $7.8 \pm 1.8$  mg/day, day 7;  $6.6 \pm 1.1$  mg/day), although differences were not statistically significant. Moreover, capsules in the control group contained 250mg of soybean oil; each capsule thus contained 0.2mg of  $\gamma$ -tocopherol. Other possible explanation is that the fluid retention in PMS is highly variable and often short lived. Our present study has a limitation, because the information such as basal hormone levels and basal body temperature in each participant was not available. Therefore, it was unclear in all women whether the "day 0" was exactly the same point in their menstrual cycle in the present study.

In the present study, the serum  $\gamma$ -tocopherol concentration significantly increased and  $\alpha$ -tocopherol concentration significantly decreased in the  $\gamma$ -tocopherol group. The interaction between  $\alpha$ - and  $\gamma$ -tocopherol metabolisms has been controversial. Leonard et al.<sup>6</sup> suggested that there is little interaction between  $\alpha$ - and  $\gamma$ -tocopherol metabolisms. On the other hand, Kiyose et al.<sup>8</sup> reported

that  $\alpha$ -tocopherol may affect  $\gamma$ -tocopherol metabolism. Morinobu et al.<sup>5</sup> also reported that high-dose administration of  $\alpha$ -tocopherol caused an increase of  $\gamma$ -tocopherol metabolism. Conversely, Clement et al.<sup>25</sup> reported that  $\gamma$ -tocopherol supplements induced a marked increase in  $\alpha$ -tocopherol concentrations in the serum and tissues. In 2005, Yoshikawa et al.<sup>13</sup> reported results similar to ours in healthy adult male volunteers. Because  $\alpha$ -TTP specifically selects  $\alpha$ -tocopherol in the liver from among all the incoming tocopherols for incorporation into VLDL and transfer to the blood,<sup>1</sup> they speculated that excess  $\gamma$ -tocopherol was taken up by the liver and combined with  $\alpha$ -TTP more than usual. As a result, the amount of  $\alpha$ -tocopherol bound to  $\alpha$ -TTP and transferred to the blood might have decreased, leading to a decrease in the serum  $\alpha$ -tocopherol concentration.

Diuretics such as loop agents and thiazides promote excretion of sodium and therefore may lead to hyponatremia as well as hypokalemia, hypochloremia, and metabolic alkalosis. Hyperkalemia is a leading complication of potassium-sparing agents such as

spironolactone. Thus, electrolyte and acid-base disorders commonly accompany diuretic use.<sup>26</sup> Agents exhibiting a prolonged and mild natriuresis (not kaliuretic) such as  $\gamma$ -CEHC might prevent electrolyte and acid-base disorders, and volume depletion with prerenal azotemia in the treatment for the volume-expanded status such as pregnancy, congestive heart failure, liver cirrhosis, and chronic renal failure.<sup>10,12</sup>

In conclusion, serum concentration of  $\alpha$ -tocopherol significantly decreased, serum concentration of  $\gamma$ -tocopherol significantly increased, and urinary excretion of  $\gamma$ -CEHC significantly increased after  $\gamma$ -tocopherol administration in young women with PMS in the present study. However, urinary sodium secretion and urine volume was not increased, and body weight did not reduced after  $\gamma$ -tocopherol administration. The degree of pretibial edema was improved but differences were not statistically significant in our small study population. Although these results suggest that  $\gamma$ -tocopherol is a precursor of prolonged natriuresis of  $\gamma$ -CEHC and may be effective for relieving edema, these function should be further validated in larger study populations, and also further studies are needed to determine the effect on other volume-expanded status such as congestive heart failure, liver cirrhosis, and chronic renal failure in humans.

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